CLAIMS

WHAT IS CLAIMED IS:

1. A method of detecting a polypeptide in a sample comprising the steps of (a) contacting a sample with a first aptamer construct and a second aptamer construct, and (b) detecting an association of the first aptamer construct, the second aptamer construct, and a polypeptide by a detection method; wherein (c) the first aptamer construct is capable of binding to a first epitope of the polypeptide and the second aptamer construct is capable of binding to a second epitope of the polypeptide, (d) the first aptamer construct comprises (i) a first aptamer that can bind to the first epitope, (ii) a first signaling oligo and (iii) a first label, and (e) the second aptamer construct comprises (iv) a second aptamer that can bind to the second epitope, (v) a second signaling oligo, which is complementary to the first signaling oligo, and (vi) a second label.

2. The method of claim 1 wherein the first and second signaling oligo each consists of at least 5 nucleotides and no more than 7 nucleotides.

3. The method of claim 1 wherein the first aptamer comprises a natural cognate binding element sequence and the second aptamer is selected using in vitro evolution.

4. The method of claim 1 wherein the first label is a fluorescence donor and the second label is a fluorescence acceptor.

5. The method of claim 1 wherein the first label is a fluorescence acceptor and the second label is a fluorescence donor.

6. The method of claim 1 wherein the detection method detects a change in fluorescence.

7. The method of claim 21 wherein the detection method is FRET.

8. The method of claim 1 wherein the first aptamer and the second aptamer are selected using in vitro evolution.

9. The method of claim 1 wherein the polypeptide does not naturally bind a natural cognate binding element sequence.

10. The method of claim 9 wherein the polypeptide is a thrombin.

11. The method of claim 10 wherein the first aptamer binds to fibrinogen exocite of the thrombin and the second aptamer binds to a fibrinogen exocite of the thrombin.

12. The method of claim 11 wherein the first label is a fluorescein.

13. The method of claim 12 wherein the detection method is fluorescence polarization.

14. The method of claim 12 wherein the second label is a dabcyl and the detection method is a detecting a change in fluorescein fluorescence intensity.

15. The method of claim 1 wherein the first aptamer construct and the second aptamer construct are joined together by a linker.

16. The method of claim 1 wherein the linker is a flexible Spacer 18 linker.

17. A method of detecting an analyte in a sample comprising the steps of (a) contacting a sample with a first aptamer construct, a second aptamer construct, and a polypeptide, and (b) detecting an association of the first aptamer construct, the second aptamer construct, the polypeptide and an analyte by a detection method; wherein (c) in the presence of the analyte, the first aptamer construct is capable of binding to a first epitope of the polypeptide and the second aptamer construct is capable of binding to a second epitope of the polypeptide, and (d) the first aptamer construct comprises a first aptamer that can bind to the first epitope, a first signaling oligo and a first label, and (e) the second aptamer construct comprises a second aptamer that can bind to the second epitope, a second signaling oligo, which is complementary to the first signaling oligo, and a second label.

18. The method of claim 17 wherein the first signaling oligo and second signaling oligo each consists of at least 5 nucleotides and no more than 7 nucleotides.

19. The method of claim 17 wherein the first aptamer comprises a natural cognate binding element sequence and the second aptamer is selected using in vitro evolution.

20. The method of claim 17 wherein the first label is a fluorescence donor and the second label is a fluorescence acceptor.

21. The method of claim 17 wherein the first label is a fluorescence acceptor and the second label is a fluorescence donor.

22. The method of claim 17 wherein the detection method detects a change in fluorescence.

23. The method of claim 22 wherein the detection method is FRET.

24. The method of claim 17 wherein the first aptamer and the second aptamer are selected using in vitro evolution.

25. The method of claim 17 wherein the polypeptide does not naturally bind a natural cognate binding element sequence.

26. The method of claim 17 wherein the polypeptide undergoes a conformational change upon binding the analyte.

27. The method of claim 26 wherein the analyte is a drug and the polypeptide is capable of binding the drug.

28. The method of claim 27 wherein the analyte is a statin drug and the polypeptide is a HMG-CoA reductase.

29. The method of claim 17 wherein the analyte is a toxin found in the environment.

30. The method of claim 17 wherein the first aptamer construct and the second aptamer construct are joined together by a linker.

31. The method of claim 30 wherein the linker is a flexible Spacer 18 linker.

32. A method of making a set of aptamer constructs, comprising a first and second aptamer construct, comprising the steps of (a) selecting a first aptamer against a first substrate, which comprises a first epitope, and selecting a second aptamer against a second substrate, which comprises a second epitope, wherein the first aptamer is capable of binding to the first epitope and the second aptamer is capable of binding to the second epitope, (b) attaching a first label to the first aptamer and attaching a second label to the second aptamer, (c) attaching a first signaling oligo to the first aptamer and attaching a second signaling oligp to the second aptamer, wherein the second signaling oligo is complementary to the first signaling oligo, and (d) such that (i) the first aptamer construct comprises the first aptamer, the first label and the first signaling oligo, and (ii) the second aptamer construct comprises the second aptamer, the second label and the second signaling oligo.

33. The method of claim 32 wherein the first substrate is a polypeptide and the second substrate is the polypeptide bound to the first aptamer, wherein the first aptamer masks the first epitope.

34. The method of claim 32 wherein the first substrate is a polypeptide or a macromolecular complex and the second substrate is the polypeptide or macromolecular complex bound to the first aptamer, wherein the first aptamer (a) is attached to the first signaling oligo or the first label and (b) masks the first epitope.

35. The method of claim 32 wherein the first substrate is a fragment of a polypeptide consisting essentially of the first epitope and the second substrate is a fragment of the polypeptide consisting essentially of the second epitope.

36. The method of claim 32 wherein the first signaling oligo and the second signaling oligo each consist of at least 5 nucleotides and no more than 7 nucleotides.

37. The method of claim 32 wherein the first aptamer comprises a natural cognate binding element sequence and the second aptamer is selected using in vitro evolution.

38. The method of claim 32 wherein the first label is a fluorescence donor and the second label is a fluorescence acceptor.

39. The method of claim 32 wherein the first label is a fluorescence acceptor and the second label is a fluorescence donor.

40. The method of claim 32 wherein the first aptamer and the second aptamer are selected using in vitro evolution.

41. The method of claim 32 comprising the step joining the first aptamer construct to the second aptamer construct with a flexible linker.

42. A bivalent aptamer construct comprising a first aptamer, a first label, a first signaling oligo, a second aptamer, a second label, a second signaling oligo and a linker, wherein the first aptamer is capable of binding to a first epitope and the second aptamer is capable of binding to a second epitope.

43. The bivalent aptamer construct of claim 42 wherein the first epitope and the second epitope are distinct and non-overlapping epitopes of a same polypeptide.

44. The bivalent aptamer construct of claim 42 wherein the linker is a flexible linker.

45. The bivalent aptamer construct of claim 44 wherein the linker is a Spacer 18.

46. The bivalent aptamer construct of claim 44 wherein the linker is a deoxythymidine polymer.

47. The bivalent aptamer construct of claim 42 wherein the first label is a fluorescence donor.

48. The bivalent aptamer construct of claim 47 wherein the second label is a fluorescence recipient.

49. The bivalent aptamer construct of claim 42 wherein the first and second signaling oligos are at least 5 nucleotides in length and no more than 7 nucleotides in length.

50. The bivalent aptamer construct of claim 42 wherein the first aptamer comprises a natural cognate binding element sequence.

51. The bivalent aptamer construct of claim 50 wherein the second aptamer is selected using in vitro evolution.

52. The bivalent aptamer construct of claim 42 wherein the first aptamer is selected using in vitro evolution.

53. The bivalent aptamer construct of claim 42 wherein the polypeptide is a thrombin, the first label is a fluorescein, the second label is a dabcyl, the first epitope is a heparin exosite, the second epitope is a fibrinogen exosite, and the linker is a Spacer 18.

54. The bivalent aptamer construct of claim 43 wherein the polypeptide is a thrombin, the first label is a fluorescein, the second label is a dabcyl, the first epitope is a heparin exosite, the second epitope is a fibrinogen exosite, and the linker is a Spacer 18.

55. A kit comprising a first epitope binding agent, to which is attached a first label, and a second epitope binding agent, to which is attached a second label, wherein (a) when the first epitope binding agent and the second epitope binding agent label bind to a first epitope of a polypeptide and a second epitope of the polypeptide, respectively, (b) the first label and the second label interact to produce a detectable signal.

56. The kit of claim 55 wherein the first epitope binding agent is an antibody.

57. The kit of claim 55 wherein the first epitope binding agent is a first aptamer construct, which comprises a first aptamer, a first label and a first signaling oligo.

58. The kit of claim 57 wherein the second epitope binding agent is a second aptamer construct, which comprises a second label and a second signaling oligo, which is complementary to the first signaling oligo.

59. The kit of claim 58 wherein the first signaling oligo and the second signaling oligo are at least 5 nucleotides in length and no more than 7 nucleotides in length.

60. The kit of claim 58 wherein the first aptamer comprises a natural cognate binding element sequence.

61. The kit of claim 58 wherein the second aptamer was selected using in vitro evolution.

62. The kit of claim 55 wherein the first label is a fluorescence donor and the second label is a fluorescence recipient.

63. The kit of claim 62 wherein the first label is a fluorescein and the second label is a dabcyl.

64. The kit of claim 55 further comprising the polypeptide, wherein the polypeptide is capable of binding an analyte.

65. The kit of claim 55 further comprising a printed set of instructions for using said kit.

66. A method of diagnosing a disease comprising the steps of (a) obtaining a sample from a patient, (b) contacting the sample with a first epitope binding agent and a second epitope binding agent, and (c) detecting the presence of a polypeptide in the sample using a detection method, wherein the presence of the polypeptide in the sample indicates whether a disease is present in the patient.

67. The method of claim 66 wherein (a) the first epitope binding agent is a first aptamer to which a first label and a first signaling oligo are attached, (b) the second epitope binding agent is a second aptamer to which a second label and a second signaling oligo, which is complementary to the first signaling oligo, are attached, and (c) the detection method is a fluorescence detection method, wherein, (d) when the first aptamer binds to the polypeptide and the second aptamer binds to the polypeptide, (e) the first signaling oligo and the second signaling oligo associate with each other, and

(f) the first label is brought into proximity to the second label such that a change in fluorescence occurs.

68. The method of claim 66 wherein the sample is selected from the group consisting of blood, urine, ascites and tissue sample.

69. The method of claim 66 wherein the patient is a human.

70. A method of diagnosing a disease comprising the steps of (a) obtaining a sample from a patient, (b) contacting the sample with a first epitope binding agent, a second epitope binding agent, and a polypeptide, which comprises a first epitope and a second epitope, and (c) detecting in the sample, using a detection method, the presence of an analyte, which is capable of binding the polypeptide, wherein the presence of the analyte indicates whether a disease is present in the patient.

71. The method of claim 70 wherein (a) the first epitope binding agent is a first aptamer to which a first label and a first signaling oligo are attached, (b) the second epitope binding agent is a second aptamer to which a second label and a second signaling oligo, which is complementary to the first signaling oligo, are attached, and (c) the detection method is a fluorescence detection method, wherein, (d) when the analyte binds to the polypeptide, (e) the first aptamer binds to the polypeptide and the second aptamer binds to the polypeptide, (e) the first signaling oligo and the second signaling oligo associate with each other, and (f) the first label is brought into proximity to the second label such that a change in fluorescence occurs.

72. The method of claim 70 wherein the sample is selected from the group consisting of blood, urine, ascites and tissue sample.

73. The method of claim 70 wherein the patient is a human.

74. A method of screening a sample for useful reagents comprising the steps of (a) contacting a sample with a first epitope binding agent and a second epitope binding agent, and (b) detecting the presence of a useful reagent in the sample using a detection method.

75. The method of claim 74 wherein the useful reagent is a polypeptide which comprises a first epitope and a second epitope.

76. The method of claim 74 further comprising the step of contacting the sample with a polypeptide, which is capable of binding an analyte, wherein the useful reagent is an analyte.

77. The method of claim 74 wherein the useful reagent is a potential therapeutic composition.

78. The method of claim 74 wherein (a) the first epitope binding agent is a first aptamer to which a first label and a first signaling oligo are attached, (b) the second epitope binding agent is a second aptamer to which a second label and a second signaling oligo, which is complementary to the first signaling oligo, are attached, and (c) the detection method is a fluorescence detection method, wherein, (d) when the first aptamer binds to the polypeptide and the second aptamer binds to the polypeptide, (e) the first signaling oligo and the second signaling oligo associate with each other, and (f) the first label is brought into proximity to the second label such that a change in fluorescence occurs.

79. The method of claim 78 wherein the useful reagent is a polypeptide which comprises a first epitope and a second epitope.

80. The method of claim 78 further comprising the step of contacting the sample with a polypeptide, which is capable of binding an analyte, wherein the useful reagent is an analyte.

81. The method of claim 78 wherein the useful reagent is a potential therapeutic composition.

82. A pharmaceutical composition comprising a bivalent aptamer construct as set forth in any one of claims 42-54.

83. A method of facilitating molecular interactions in a sample comprising the step of administering to the sample a bivalent aptamer as set forth in any one of claims 42-54, wherein the first epitope and the second epitope are on separate molecular entities and the first epitope and second epitope are brought into close proximity to effect a molecular interaction.

84. The method of claim 83 wherein the sample is selected from the group consisting of cell, tissue, cerebral spinal fluid, blood, in vitro reaction mixture, and environmental system.

85. A method of detecting a polypeptide in a sample comprising the steps of (a) contacting a sample with a first molecular-recognition construct and a second molecular-recognition construct, and (b) detecting an association of the first molecular-recognition construct, the second molecular-recognition construct, and a polypeptide by a detection method; wherein (c) the first molecular-recognition construct is capable of binding to a first epitope of the polypeptide and the second molecular-recognition construct is capable of binding to a second epitope of the polypeptide, (d) the first molecular-recognition construct comprises (i) a first epitope-binding agent that can bind to the first epitope, (ii) a first signaling oligo and (iii) a first label, and (e) the second molecular-recognition construct comprises (iv) a second epitope binding agent that can bind to the second epitope, (v) a second signaling oligo, which is complementary to the first signaling oligo, and (vi) a second label.

86. The method of claim 85 wherein the first epitope binding agent is an aptamer.

87. The method of claim 86 wherein the second epitope binding agent is an aptamer.

88. The method of claim 86 wherein the second epitope binding agent is an antibody.

89. The method of claim 86 wherein the second epitope binding agent is a double stranded polynucleotide containing binding site for the polypeptide.

90. The method of claim 85 wherein the first epitope binding agent is an antibody.

91. The method of claim 90 wherein the second epitope binding agent is a second antibody.

92. The method of claim 90 wherein the second epitope binding agent is a double stranded polynucleotide containing binding site for the polypeptide.

93. The method of claim 85 wherein the first epitope binding agent is a double stranded polynucleotide containing first binding site for the polypeptide.

94. The method of claim 93 wherein the second epitope binding agent is a double stranded polynucleotide containing second binding site for the polypeptide.

95. The method of any one of claims 85 through 94 wherein the detection method is selected from the group consisting of plasmon resonance, fluorescence resonance energy transfer ("FRET)), FCCS, fluorescence quenching, fluorescence polarization, production of a colored product, chemiluminescence, scintillation, bioluminescence, and luminescence resonance energy transfer.

96. The method of claim 95 wherein the detection method is luminescence resonance energy transfer.

97. The method of any one of claims 85 through 96 wherein the polypeptide is thrombin or cAMP response element binding protein ("CRP").

98. The method of any one of claims 85 through 97 wherein the sample is selected from the group consisting of blood, urine, ascites, cellular sample and tissue sample.

99. A molecular beacon comprising a first molecular-recognition construct and a second molecular-recognition construct; wherein (a) the first molecular-recognition construct is capable of binding to a first epitope of a polypeptide and the second molecular-recognition construct is capable of binding to a second epitope of the polypeptide, (b) the first molecular-recognition construct comprises (i) a first epitope-binding agent that can bind to the first epitope, (ii) a first signaling oligo and (iii) a first label, and (c) the second molecular-recognition construct comprises (iv) a second epitope binding agent that can bind to the second epitope, (v) a second signaling oligo, which is complementary to the first signaling oligo, and (vi) a second label.

100. The molecular beacon of claim 99 wherein the first epitope binding agent is an aptamer.

101. The method of claim 100 wherein the second epitope binding agent is an aptamer.

102. The method of claim 100 wherein the second epitope binding agent is an antibody.

103. The method of claim 100 wherein the second epitope binding agent is a double stranded polynucleotide containing binding site for the polypeptide.

104. The method of claim 99 wherein the first epitope binding agent is an antibody.

105. The method of claim 104 wherein the second epitope binding agent is a second antibody.

106. The method of claim 104 wherein the second epitope binding agent is a double stranded polynucleotide containing binding site for the polypeptide.

107. The method of claim 99 wherein the first epitope binding agent is a double stranded polynucleotide containing first binding site for the polypeptide.

108. The method of claim 107 wherein the second epitope binding agent is a double stranded polynucleotide containing second binding site for the polypeptide.